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The alimentary tract of arostrate, dwarfish males of the aphid genus *Stomaphis* (Insecta, Hemiptera)

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Abstract

Dwarfish males are a relatively common phenomenon in animals, occurring in various taxa, most often when females conduct a sedentary life mode. Such males, beside smaller size, exhibit a series of morphological and anatomical alterations, mostly structural reductions. Here we present the case of the alimentary tract of dwarfish males of the aphid genus *Stomaphis* where, despite it being structurally normal, it does not serve its original function due to a lack of mouthparts. Cross-sections through mounted specimens revealed also that nervous and reproductive systems are fully developed. The question arises as to whether such a system may be considered vestigial, or an exaptation with secondary function shifted to play new primary function. Because the aphid genus *Stomaphis* is known from the fossil record to have existed for at least ca. 14 My and all of its species have dwarfish, arostral males, this indicates that it may be too short a period for reduction of the whole system. It also raises questions about the mechanisms of loss of the primary functionality of the whole physiological unit, and the influence of such modification on the evolution of its phylogenetic successors. Secondary functions of the tract are speculated to be responsible for its endurance.

Keywords

evolution – exaptation – mutualism – natural selection – sexual dimorphism

Introduction

Male dwarfism is an interesting evolutionary phenomenon, which evolved independently in many various groups of animals such as rotifers (Ricci & Melone, 1998), annelids (Rouse et al., 2015) or arthropods (Danielson-François et al., 2012) including aphids (Wieczorek, 2008). It occurs when the size of male does not exceed 50% of the size of female body (Vollrath, 1998) and is often associated with heterochrony or a reduction in the structure and functionality of organs (Vollrath, 1998), with many of them thus becoming vestigial (Hall, 2003). In aphids organ reductions include the lack of wings, a reduced number of testes or loss of mouthparts (Wieczorek, 2008; Wieczorek & Świątek, 2009).

Considering loss of mouthparts, an interesting case is that of the aphid genus *Stomaphis* where, apart from dwarfish males, female gigantism is also present (Depa et al., 2014, 2015). These are tree trunk feeding aphids, obligatorily dependent on their mutualistic relationship with ants. Most species live in bark crevices covered with soil by ants or inside ant chambers built in cork tissue of tree bark. As an adaptation to this kind of feeding location, females have their mouthparts up to twice the body length. As well as being significantly smaller than females, males of *Stomaphis* are characterised also by frequent aberrations in the structure of appendages and, as a rule, are arostrate, which precludes feeding (fig. 1A-C). Males moult 3 times (3 instars and adult stage) and during all larval stages they do not grow and no feeding behaviour is known, as also larvae in all stages are devoid of mouthparts (Depa et al., 2015). It has been speculated that the loss of male mouthparts is an adaptation to copulation (Dixon, 1998). However, the female rostrum was observed to be moved aside of the body during copulation, so the concept that the male mouthparts are reduced to facilitate mating has been dismissed by Depa et al. (2015). They proposed a new explanation, claiming that the loss of mouthparts is a degenerative process resulting from increased inbreeding within aphid populations having a cryptic and sedentary mode of life under the strong influence of mutualistic ants. Loss of mouthparts seems to be linked to male dwarfism in *Stomaphis* which may result from this loss, as the lack of feeding should definitely disable uptake of nutrients necessary for growth.

The absence of any traces of stylets (Depa et al., 2015; fig. 1B, C) indicates a permanent loss of feeding ability as other modes of feeding e.g., through trophallaxis are unknown in aphids. In line with the existence of male aberrant appendages, such loss of feeding ability raises questions about the possible internal equivalent of losing the ability to feed. The general hypothesis was that, as a consequence of losing the external feeding organs, the internal organs responsible for nutrition (i.e., the alimentary tract) would also be reduced. A high number of fat cells was expected as being responsible for post-embryonic growth, while well-developed reproductive organs were recorded in earlier research (Wojciechowski, 1977). This study presents the results of an investigation into the structure of the internal organs of dwarfish males of *Stomaphis*, with special attention given to the unexpected occurrence of a complete alimentary tract.

Material and methods

Research was focussed on aphids from the genus *Stomaphis* Walker, specifically *Stomaphis* (*Parastomaphis*) *graffii* Cholodkovsky, 1894 and *Stomaphis* (*Stomaphis*) *quercus* (Linnaeus, 1758). The male specimens (fig. 1A) were collected in 2017-2018 from southern Poland (Upper Silesia; Katowice, Piekary Śląskie) to

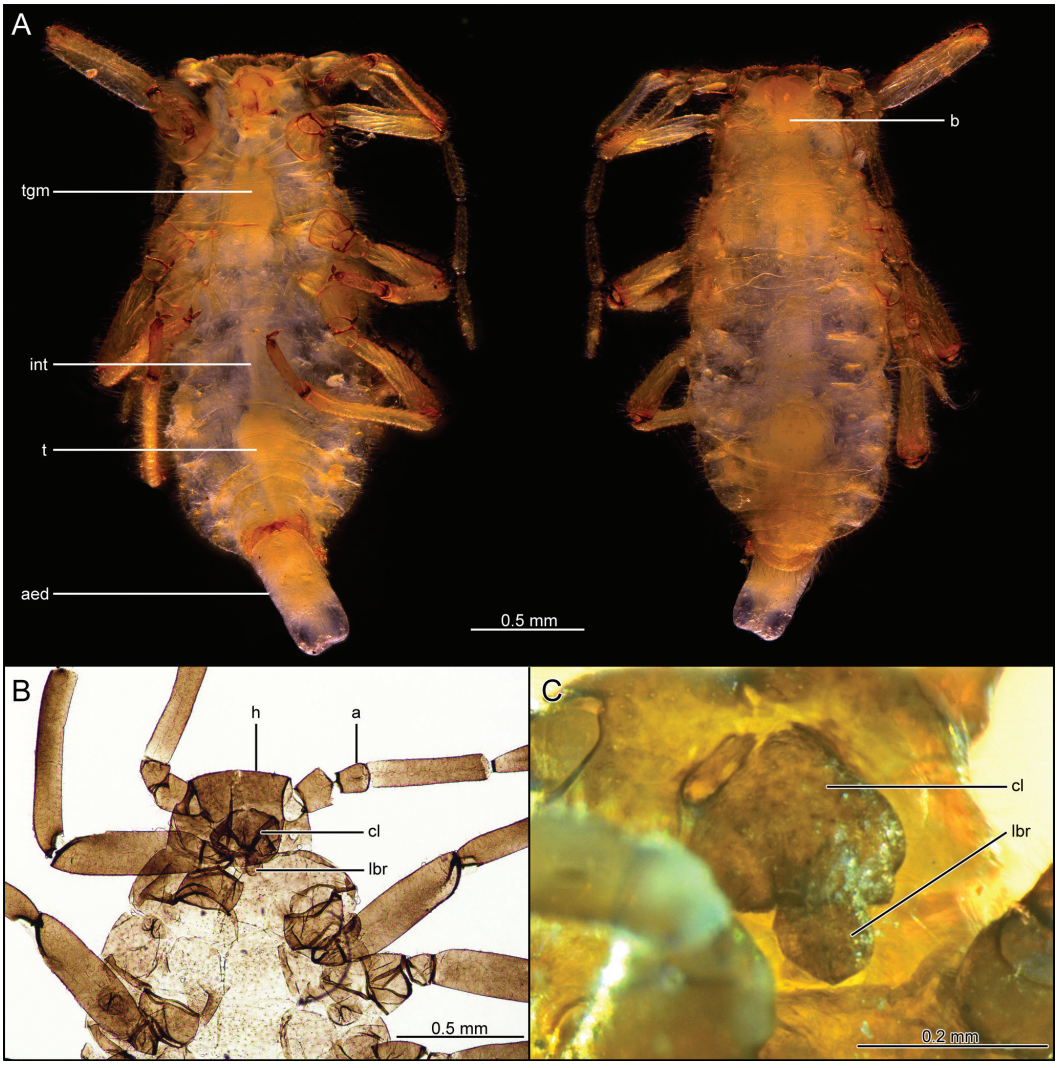

FIGURE 1 Body of an adult male of *S. graffii*: ventral and dorsal (A) view, with aedeagus partly everted; head in microscopic slide (B) with reduced mouthparts and their close-up in macroscopic view (C).

Eppendorf tubes with 2.5% glutaraldehyde solution and 70% ethanol solution. Aphids were collected from *Acer pseudoplatanus* (Linnaeus, 1753) in the case of *S. graffii* and from *Quercus robur* (Linnaeus, 1753) in the case of *S. quercus*. A total of 15 males and several oviparous females was collected for identification and study. Females were mounted singly on microscope slides using the method described by Kanturski and Wieczorek (2012) and the female specimens were identified by the senior author using the key presented in Depa et al. (2015).

The main method used in this work were histological preparations. We used four 2nd instar larvae and four adult males of *S. graffii* and two 2nd instar larvae and two adult males of *S. quercus*. The material was fixed in 2.5% glutaraldehyde in a 0.1 M phosphate buffer (pH = 7.4) at room temperature. After washing in a 0.1 M phosphate buffer, the material was fixed for 2 h in a 1% OsO_4 in phosphate buffer.

Then, the material was washed in a phosphate buffer and in a graded series of ethanol, which were replaced by acetone. Next, the material was embedded in epoxy resin. Semi-thin sections (1.0 μm thick) were cut on a Leica Ultracut ultramicrotome and stained with 1% methylene blue in a 1% sodium biborate solution at room temperature for 30 s. Colour images of the aphids were captured with following equipment: Nikon Eclipse E-600 biological microscope with Nikon DS-Fi2 digital camera and NIS Elements 4.10 software; Leica M205C (stereomicroscope), Leica LED5000 HDI (high diffuse dome illumination), Leica DFC495 (camera) and Leica application suite 4.9.0 (software); Nikon SMZ 1500 stereomicroscope with Schott EasyLED Ringlight Plus and EasyLED Spotlights illuminators, Nikon DSFi3 digital camera and NIS Elements 4.10 software, Image Composite Editor (panoramic image stitcher) and Adobe Photoshop CS6 graphic editor. For preparation of images from stereoscopic microscopes the specimens, preserved in 70% ethanol, were placed in a watch glass. Photographs of whole specimens were taken using a dark field technique, while photographs of the clypeus and internal organs were taken using a bright field technique. In addition to the newly-collected specimens, the microscopic slides with histological preparations of *Stomaphis quercus* and *Maculolachnus submacula*, made by and used for his research by Wojciechowski (1977) and preserved in excellent condition in the collection of the Faculty of Natural Sciences of the University of Silesia in Katowice, were also examined.

The following field observations, referring to fig. 9 were included:

a. specimen 984: collected from *Acer campestre* on 08.10.2019 in Monks Wood National Nature Reserve, Sawtry, Cambridgeshire, UK; 52-24-24.54N 000-14-57.99W; leg. J. Hodgson;
b. specimen 994B: collected from *Acer campestre* on 16.10.2019 in Brampton Wood Nature Reserve, Brampton, Cambridgeshire, UK, 52-19-8.60N 000-16-35.78W; leg. J. Hodgson).

Additional analysis comprised measurements of body length of males of *S. graffii*, *S. longirostris* and *S. quercus* in particular developmental stages (3 larval stages and adult), to detect any changes in size caused by feeding. Unfortunately, unequal number of particular instars (1st – 4 specimens, 2nd – 12, 3rd – 11, adult – 11) was available in the entomological collection, so the results must be taken with certain caution. The base for measurements were mounted specimens on microscopic slides available in the entomological collection of the former Department of Zoology of the University of Silesia in Katowice (DZUS).

List of abbreviations

The following abbreviations for anatomical structures are used in the figure captions (following Ponsen, 1987): **a** – antenna, **aed** – aedeagus, **b** – brain, **cb** - central body, **cl** – clypeus, **coeg** – circumoesophageal ganglion, **ejd** – ejaculatory duct, **fc** – fat cell, **fch** – filter chamber, **h** – head, **hg** – hindgut, **int** – intestine, **lbr** – labrum, **mf** – muscle fibre, **msg** - mesothoracic ganglion, **mtg** - metathoracic ganglion, **oen** – oenocyte, **oes** – oesophagus, **oev** – oeasophageal valve, **pg** - prothoracic ganglion, **php** - pharyngeal pump, **pm** – paramere, **r** – rectum, **sog** - suboesophageal ganglion, **st** – stomach, **t** – testis, **tb** - tentorial bar, **tf** – testicular follicle, **tgm** - thoracic ganglionic mass, **vd** – vas deferens, **vnc** – ventral nerve cord.

Results

The results of measurements of body length in particular developmental stages are presented in table 1. They show no measurable increase of body length, as mean body length of 1st

TABLE 1 Mean body length of males of *Stomaphis* spp. in particular developemental stages (n – number of specimens measured, *SD* – standard deviation in series)

	1st instar (n = 4)	2nd instar (n = 12)	3rd instar (n = 11)	adult (n = 11)
mean body length [mm]	2.84	2.78	2.77	2.72
SD	0.18	0.18	0.09	0.17

instar larva was 2.84 ± 0.18 mm and adult 2.72 ± 0.17 mm.

Within the body cavity of *Stomaphis* males the following structures are visible: alimentary tract, nervous system, reproductive system (figs 2, 3A, B), locomotor system in the form of single muscle fibres, tracheae and dorsal vessel. In larval stages most of the body cavity is filled with fat body cells and multiple individual oenocytes, placed dorso-laterally. In adults the size of fat cells is reduced – in 2nd instar larvae the fat body cells measure on average 64.16 ± 12.15 μm in diameter while in adults they measure only 33.71 ± 7.80 μm in diameter.

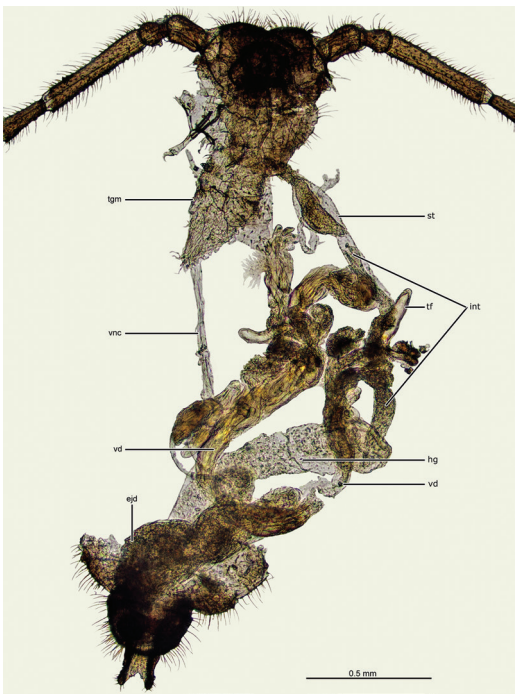

FIGURE 2 General topology of the internal organs of adult male of *S. graffii* extracted from the body: all three main systems (digestive, nervous and reproductive) clearly discernible.

Alimentary tract

The dwarfish male of *Stomaphis* is characterised by the absence of the *labium* and mandibulary and maxillary stylets. In most cases only the *clypeus* and *labrum* occur (fig. 1B, C). Despite the absence of mouthparts, many elements of the sucking pump are present within the head (fig. 3C, D), consisting of the pharyngeal duct and divaricator muscles attached to the pharyngeal pump; however, they are very variably developed, being better developed in *S. quercus* than in *S. graffii*. Retractor muscles, normally attached to the stylet bases and to the tentorial bar, are absent in both species.

The foregut (*oesophagus*) (figs 3C, 4A) is a uniformly thin (14-19 μm in diameter), 160-270 μm-long tube that runs posteriorly above and beyond the tentorium, invaginating into the stomach anteriorly as a weakly developed oesophageal valve, somewhere in the mesothorax.

The midgut consists of an elongated, somewhat dilated sac-like stomach (fig. 2) about 70-90 μm in maximum diameter and ca. 300 μm long; exact measurement is difficult because the distal part of the stomach is often bent laterally and passes smoothly into the intestine (figs 3C, 4A, B, C). The anterior part of the stomach consists of cuboidal cells, while the middle and distal part consists of strongly vacuolated columnar cells. The intestine is thin, about 50-85 μm in diameter and elongated, with one loop passing usually below the stomach and later also a second loop

beyond the stomach (figs 3B, 4B, C). Along its whole length it is built of vacuolated, cuboidal cells. It seems that the length and number of loops is variable, but generally this part of the alimentary tract is simple, not reaching proximally further than the anterior edge of the stomach and posteriorly not further than the anterior part of the hindgut. No traces of any kind of filter chamber are visible, and along most of its length the intestine does not adhere to any other part of the alimentary tract. Some variability in the form and position of the foregut and midgut is observed, with the *oesophagus* and stomach sometimes moved slightly laterally to the body axis and the loops of intestine sometimes oriented slightly laterally to the stomach.

In the anterior part of the abdomen, in abdominal segment I or II, the intestine passes into the hindgut (figs 2, 3A). This part of the

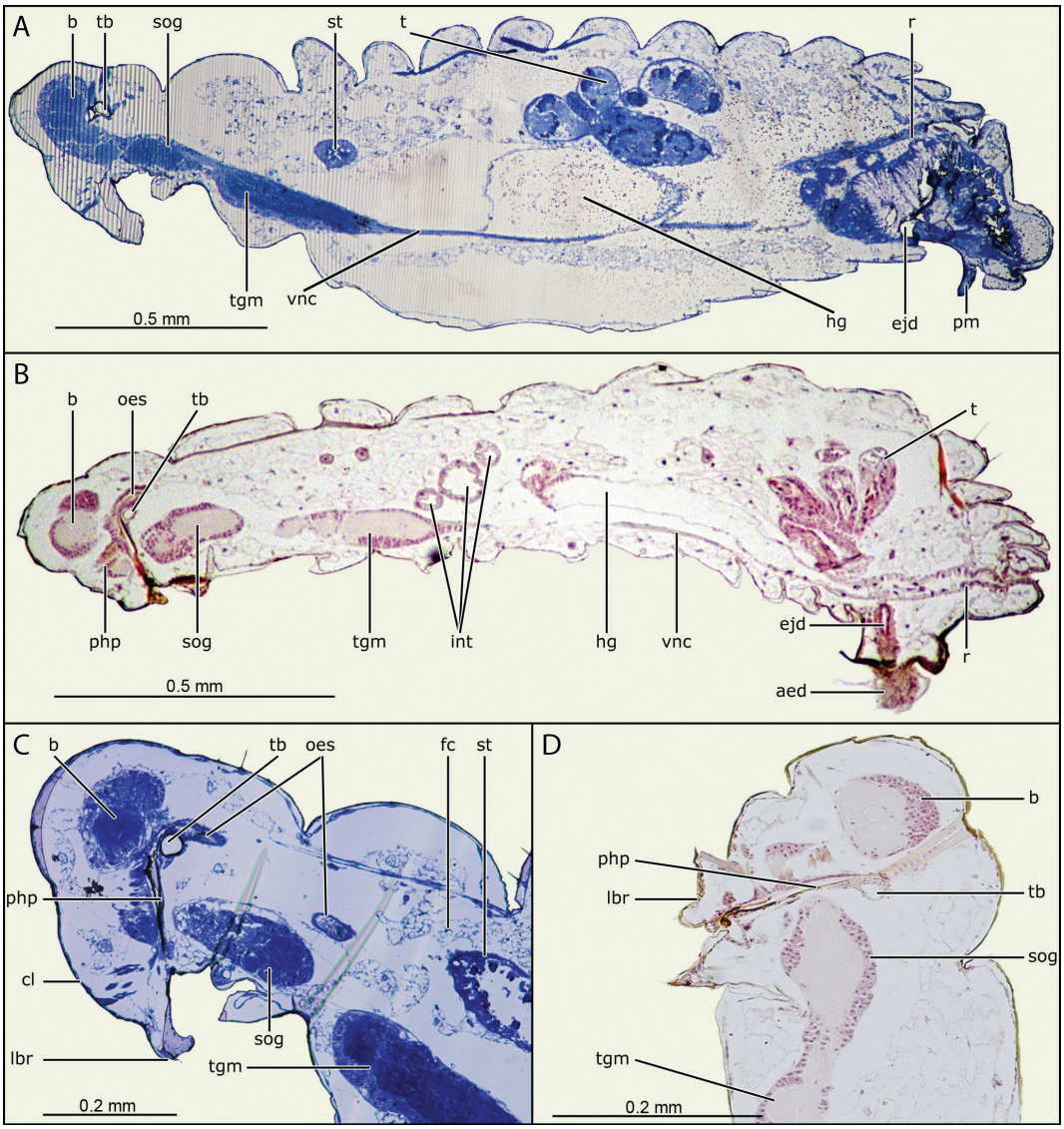

FIGURE 3 Cross sections of adult males of *S. graffii* (A, C) and *S. quercus* (B, D) showing the total body sections (A, B) and section through head and prothorax (C, D).

alimentary tract is placed ventrally in the abdomen, usually in its middle to posterior parts, below the reproductive system. The hindgut (figs 3B, 4B) is a sac-like structure, with its anterior part built of spherical, strongly vacuolated cells, partly breaking off to the lumen, while the posterior part has thin walls built of a single layer of squamous cells (fig. 4B, C, D, E). It is very variable in diameter, but is broadest at the beginning (200-300 μm), narrowing gradually towards the distal part of the abdomen where it forms a thin canal (590-760 μm long) above the ejaculatory vesicle, and ending with the rectum and anal opening (figs 4E, 5D). In larval stages, this part of the alimentary tract is placed more ventrally and is of smaller diameter, seemingly being flattened under a mass of fat body cells located in the central part of the abdomen.

Nervous system

The typical nervous system of aphids consists of four main structural parts: the brain or supraoesophageal ganglion, occupying most of the head capsule; the suboesophageal ganglion, attached to the brain by connectives; and the thoracic ganglionic mass, lying behind the suboesophageal ganglion and terminating in the ventral nerve cord (fig. 3A).

The head part of the nervous system resembles in shape an elongated ellipse and in horizontal section is bilobed (fig. 4A). The brain is divisible into three regions; *protocerebrum, deutocerebrum* and *tritocerebrum*, all of them visible and distinguishable in the cross sections. The short frontal nerves, which connect to the frontal ganglion, originate from the central, inner regions of the tritocerebral lobes. Placed slightly more ventrally is the pharyngeal ganglion, which innervates the pharyngeal dilator muscles and is connected through commissures with the suboesophageal ganglion in the posterior part of the head (fig. 5A). It further passes into the thoracic ganglia, subdivided ultrastructurally into prothoracic, mesothoracic and metathoracic

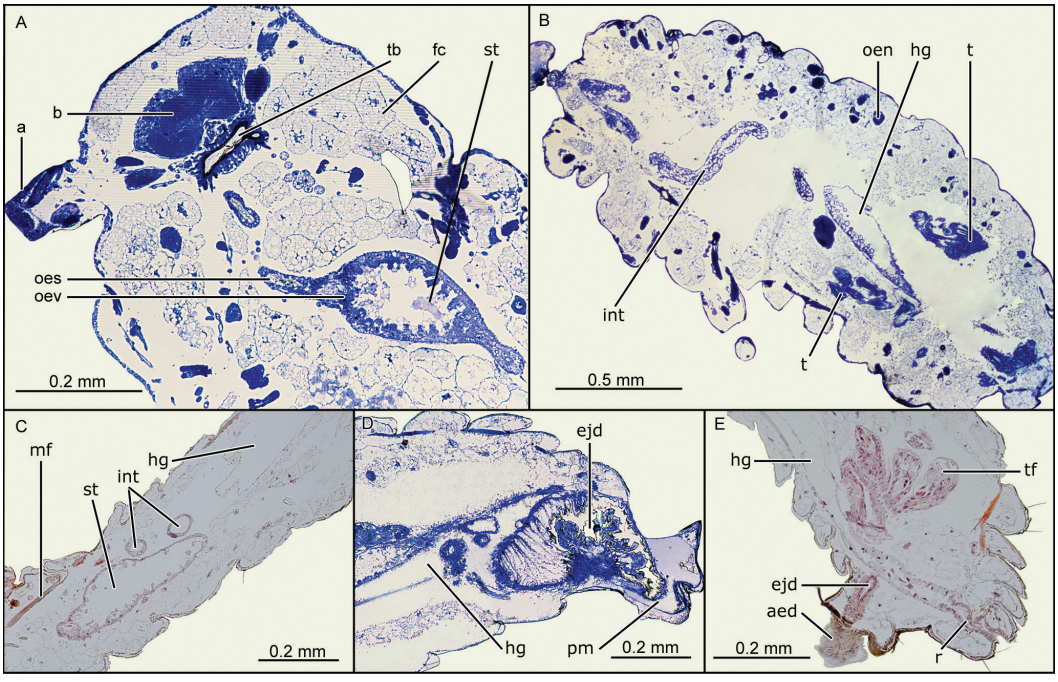

FIGURE 4 Details of the internal structure of adult males of *S. graffii* (A, B, D) and *S. quercus* (C, E).

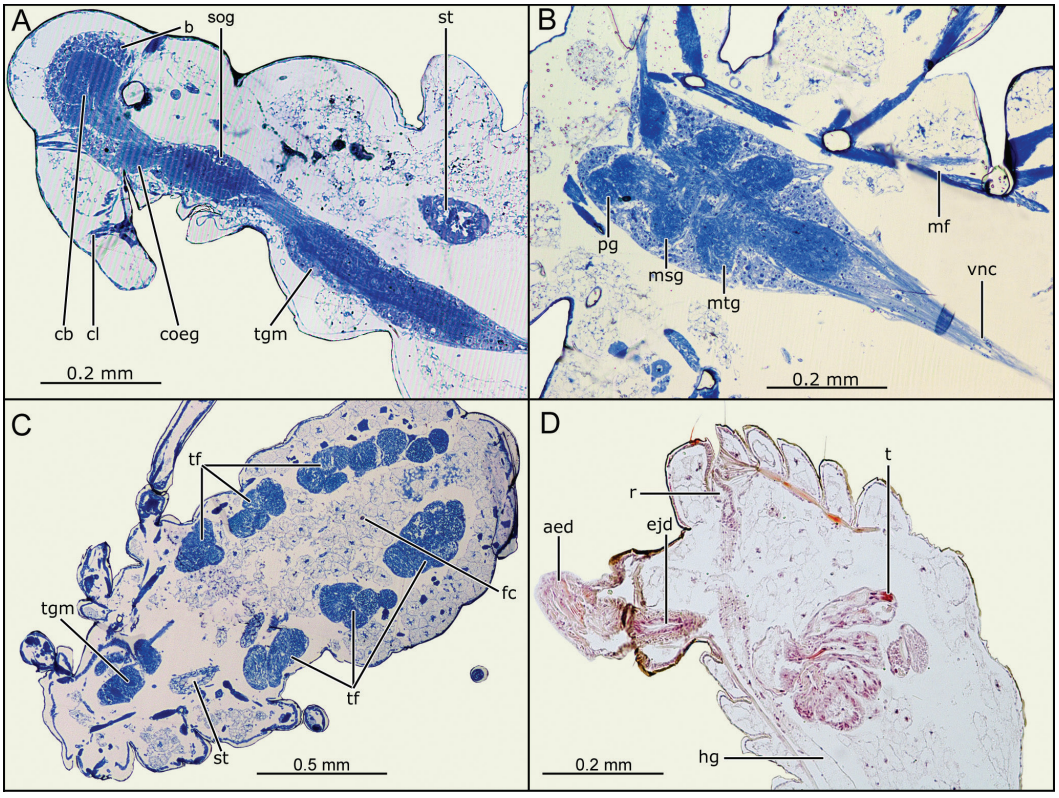

FIGURE 5 Details of the nervous (A, B) and reproductive (C, D) systems of adult male of *S. graffii* (A, B), 2nd instar larva of *S. graffii* (C) and adult male of *S. quercus* (D).

ganglia, fused into a single ganglionic mass (fig. 5B), from which nerves innervating the legs arise. From the thoracic ganglion, somewhere in the metathorax, a ventral nerve cord arises, passing through the abdomen to its distal part and the ejaculatory duct.

Reproductive system

The reproductive system of *Stomaphis* is shaped similarly to other Lachninae. Each testis consists of testicular follicles, four in *S. quercus* and three in *S. graffii*, about 100-200 μm in diameter and 200-600 μm in length. In larvae, testicular follicles are placed dorsolaterally, with the middle part of the abdomen filled with fat cells (fig. 5C). In adults, the mature follicles are located in the middle – posterior part of the abdomen (fig. 3A). In adult males two *vasa deferentia*, about 400-600 μm long, join at the beginning of their course and run independently backwards and down the abdomen to the ejaculatory duct (fig. 2). In larval stages, the ejaculatory duct resembles a cyst and should properly be called the ejaculatory vesicle; it transforms in the adult into the ejaculatory duct. As in all Lachninae, there are no accessory glands. The ejaculatory duct has strongly wrinkled or folded walls (figs 3A, 4D) that probably increase its surface area, allowing it to be everted during copulation as a membrane called the *aedeagus* (figs 4E, 5D), within which are the orifices of the *vasa deferentia*. Functioning of the duct in this way explains why spermatozoa are stored in testes before copulation – evertion of the duct places the orifices of the *vasa deferentia* directly at the end of the *aedeagus* when it is inserted into the copulatory organs of the female.

Field observations

Additional field observations of colonies of *S. graffii* showed that some males are able to excrete single, small drops of translucent fluid of unknown composition and destiny (fig. 9).

Discussion

The labium and stylets in most male individuals are completely lacking, with only the clypeus present, and infrequently the residual last segment of the labium, without any traceable remains of stylets (Depa et al., 2015). Some aberrations of other appendages are also recorded, except for the external reproductive organs (Depa et al., 2015). In this respect, also the internal organs show an unequal degree of underdevelopment. The most important is the presence of an almost normally developed alimentary tract, with all of the consecutive sections from the oesophagus to an unobstructed anal duct, while it was suspected that the alimentary tract would be far more reduced or absent. The theoretical possibility that males lacking stylets feed via anal trophallaxis from females is an unknown phenomenon in aphids. On the other hand, the presence of a gut is an evolutionarily old and crucial trait, so its retention in a relatively young group (recorded from the Miocene – Wegierek & Mamontova, 1993) should not be surprising. It is possible that the alimentary tract of the *Stomaphis* male did not undergo significant reduction during the evolution of this genus. Also, an observed asymmetry of the midgut may be symptomatic of vestigial organs (Crespi & Vanderkist, 1997; Tague, 1997) when there is a lower selective pressure on functionality, although asymmetry of gut is quite often in many feeding animals (Burn & Hill, 2009). Comparison of its structure with that of some other aphid taxa e. g. Adelgidae or Aphidinae (Ponsen, 1990; fig. 6) shows that the studied alimentary tract is just a shortened version of the basic model of many other aphids and is more similar to that of unrelated taxa than to one of its females. Within the large body of *Stomaphis* females there exists a complicated system of coiled ducts of intestine (Klimaszewski & Wojciechowski, 1979), at

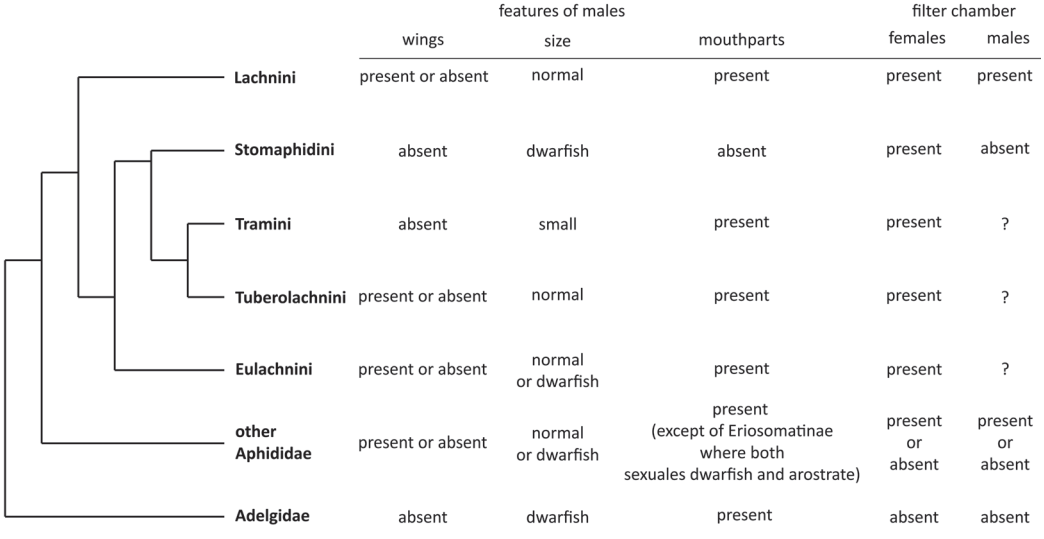

FIGURE 6 Features of males and of the alimentary tract of Lachninae aphids compared to other Aphidomorpha; phylogeny of Lachninae after Chen et al. (2016).

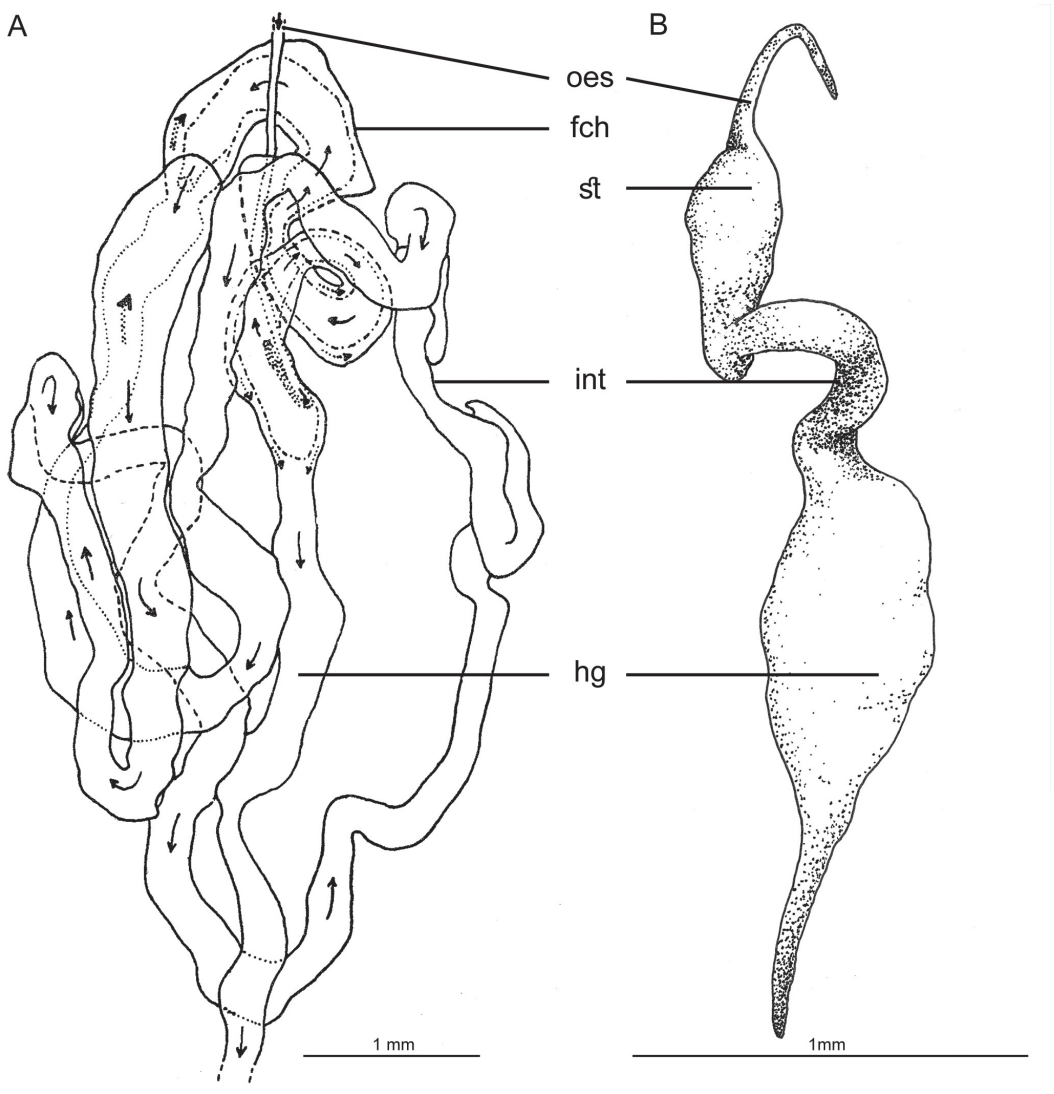

FIGURE 7 Schematic draft of alimentary tract of adult female of *S. quercus* (A) and adult male (B) of *S. graffii* (A – after Klimaszewski & Wojciechowski, 1979, altered).

one point one passing through another and creating the so called filter chamber (serving osmoregulation, Shakesby et al., 2009) (fig. 7A), similar to that present in related aphids (including some males) of the subfamily Lachninae (Klimaszewski et al., 1976) (figs 6, 8B, C). In the *Stomaphis* male no significant similarity to any kind of filter chamber can be traced (fig. 7 B). Usually in aphids males have an alimentary tract that is very similar to the conspecific females (Ponsen, 1990). The similarity of the *Stomaphis* male system with other aphids and its dissimilarity with congeneric females indicates that perhaps during the course of evolution it was not the male's alimentary system that reduced but female's that underwent expansion and modification. Unless the reduction of multiple intestinal loops in *Stomaphis* males, present in females, to a short and simple intestine is an anatomical equivalent of the loss of mouthparts.

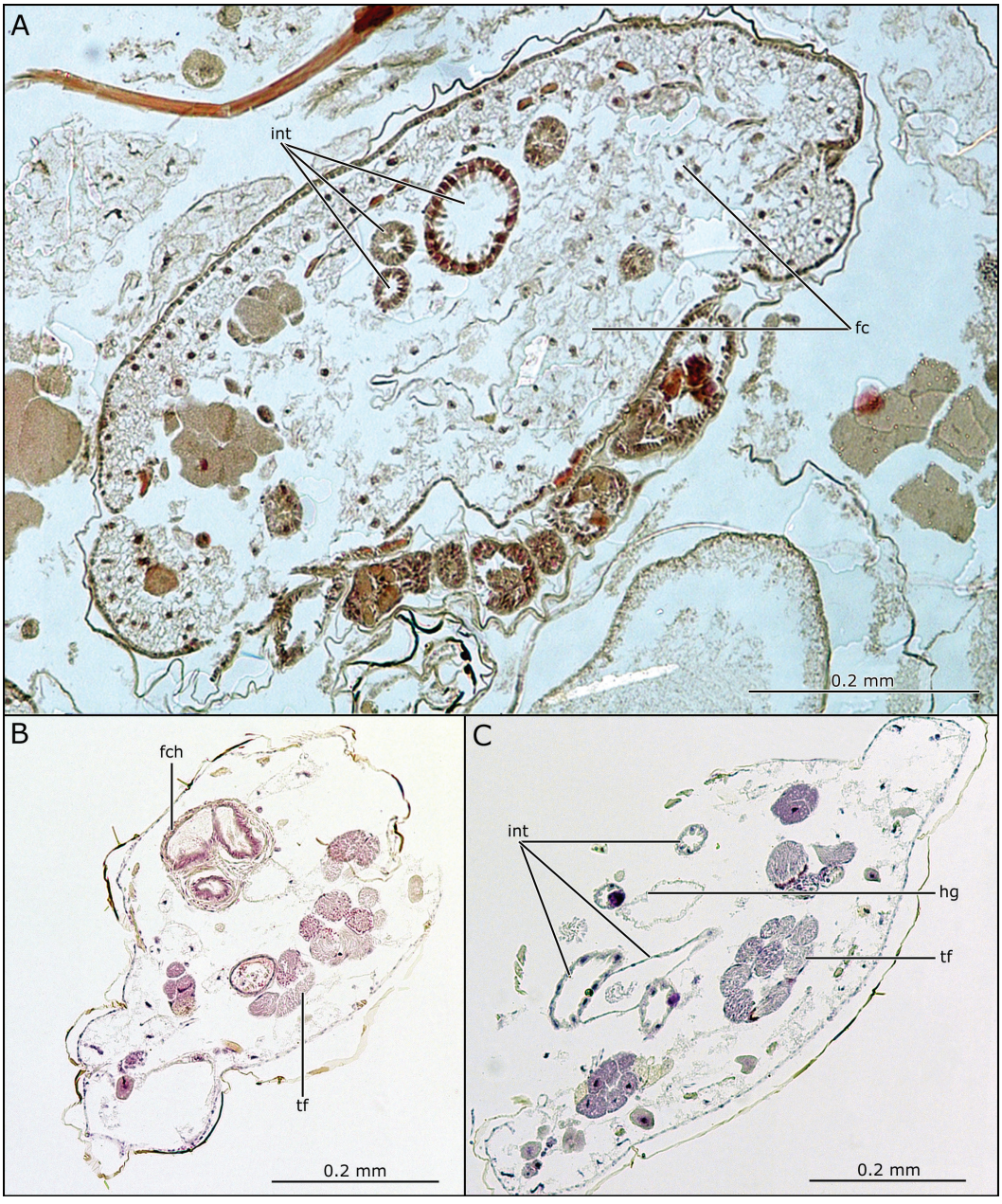

FIGURE 8 Cross section through female embryo of adult viviparous female of *S. graffii* (A), and through adult male of *Maculolachnus submacula* (B, C).

Taking into account recent views on phylogenetic relationships within Lachninae (fig. 6) and the various size of males in the related genera e.g., *Trama* or *Lachnus* (Blackman et al., 2001; fig. 6), both of which have functioning mouthparts, the male in *Stomaphis* has been relatively small and most probably wingless since the divergence of this genus. Nevertheless, in all other Lachninae species, males have mouthparts and are actively feeding. It is therefore reasonable to suspect a causal link between absence of mouthparts

and dwarfism in *Stomaphis* males. However, the reason for dwarfism is unobvious. Previous studies suggest that sedentary life mode of females and the low density of females promotes evolution of male dwarfism (Vollrath, 1998). In *Stomaphis*, female density is probably lower than in other aphids and sedentary life mode is also a clue, because living in confined spaces in ant chambers significantly decreases movements of females. However, living in confined spaces leads to higher availability of females and should counteract male dwarfism through increased competition between males. Also, in many taxa with dwarfish males these are sort of ectoparasites joined with females (angler fish, Cycliophora, Echiuroidea, barnacles), often in larger numbers (barnacles, Echiuroidea) (Vollrath, 1998; Yusa et al., 2012). In *Stomaphis*, to a certain degree, sexual selection for male dwarfism could have had effects on mothers, investing in fat cells of sons during their embryonic development, if sons cannot feed after birth. Absence of mouthparts in larvae with a high enough number of fat body cells at birth (Ponsen, 1987) (e.g., the female embryo of *S. graffii* filled with fat cells, fig. 8A), could have little impact on their ability to mature and develop basic reproductive organs. In this respect, there are two gaps in our knowledge of *Stomaphis* biology: first one is the sex ratio between males and oviparous females, and the second one is certainty, that sexuparous females in late summer bear both sexes. Knowing the sex ratio would provide crucial data on possible female investment in male or female progeny, e.g., more small males and fewer large oviparous females, or vice versa. Some weak evidence from mating observations (Depa et al., 2015) with a few (2-3) males sitting on a single oviparous female may suggest that the sex ratio is male-biased. This however cannot be supported by serious numerical data because of difficulties in collecting such data. The second hypothesis for the evolution of male dwarfism and loss of mouthparts results from the possibility of female investment into bearing many small sons instead of a few oviparous daughters. As a tree trunk feeder, *Stomaphis* requires very long mouthparts to pierce through the bark and feed, and this further requires a large body size, to invert the long rostrum within the body cavity while exposing the stylets. Thus, bearing larger sons with mouthparts might decrease the number of produced oviparous daughters. Unless there are no sexuparous females but only androparous and gynoparous, having different strategies to produce either sons or daughters. This however strongly contradicts the well-established fact of constancy of life cycle within particular aphid families (Shaposhnikov, 1985), and so far studied Lachninae show only the existence of sexuparous females. It can be only speculated that the selective pressure was to maintain reproductive abilities and not necessarily locomotive or olfactory abilities, as the sedentary life mode of large females within the confined living space of the ant chamber, meant that reproductive (oviparous) females were within walking distance (Depa et al., 2015). The characteristic feature of the male larva is a large amount of fat cells filling out the whole body, degenerating in consecutive moults and visibly diminished in adult specimens. This suggests that much of the post-embryonic development of the internal organs is facilitated by retrieving nutrients from fat cells and not from feeding, also supported by the fact that we do not observe any growth during the juvenile stages (table 1).

Despite an unproblematic access to females, males retained a nervous system almost unchanged and even relatively massive – the brain and thoracic ganglia are both present and well developed. The main nerve is a ventral nerve cord passing throughout the body and connecting to the copulatory organs. Sensory nerves are also present, as males of *Stomaphis* have eyes, although reduced to a

few ommatidia and triommatidium, and primary rhinaria on their antennae. The importance of this system for proper reproductive effectiveness may have maintained its structure and functionality.

The least modified is the reproductive system, which is the most voluminous and shows a structure typical of previously studied *S. quercus* (Wojciechowski, 1977). During its development, testes move from anterio-lateral parts of the body towards the medio-distal part of the abdomen, where the *vasa deferentia* connect with the ejaculatory vesicle (duct) which, by protruding, becomes a functional aedeagus. The relatively large aedeagus constitutes about one third of the body length of the adult male, and is membranous to fit the copulatory segment of the large female's reproductive tract.

In comparison to other dwarfish males known in aphids, the males of *Stomaphis* are characterized by increased reductions and aberrations in external morphology. This is contrary to e.g., *Pseudessigella* males, where well-developed appendages and sensilla are present (Kanturski et al., 2017) along with fully developed reproductive (external and internal) organs, and contrary also to *Anoecia corni* or *Glyphina betulae*, in which the number of testicular follicles is reduced (Wieczorek, 2008; Wieczorek & Świątek, 2009). It seems that almost all of the parts of the alimentary tract are fully developed. The lack of a filter chamber, which may be regarded as the single main structural reduction in this system, and shortened intestine would probably not inhibit its functionality in the case of such a small male. Such a simple alimentary tract functions in many aphid genera (Ponsen, 1990). Unfortunately, the structure of the male alimentary tract in the closest related genus, *Trama*, cannot be studied as males of this genus are rarely found (Blackman et al., 2001); however, apart from a certain morphological similarity to *Stomaphis*, males of *Trama* have a long and well-developed rostrum. If long-term selection was involved, the alimentary tract should be far more reduced, comparably to the mouthparts. It seems, however, that the processes of embryonic growth of the alimentary tract are working and this may be due to lack of selective pressure or selective pressure for different function. In a plausible course of dwarfing, original small and wingless males probably already depended on nutrients provided by their mothers during embryonal growth. Loss of mouthparts had no adverse effect on the reproductive condition of the male, but if passed down to offspring, this trait could lead to further dwarfism. This process could be exhibited in its first stage by, for example, loss of the filter chamber, development of which in non-feeding males would be unnecessarily costly, as osmoregulation would be redundant. The energy cost of male development (including semen) was transferred to females, possibly to balance their sons' inability to feed.

It is worth noting that the lack of stylets in both subgenera that diverged from one another sometime in or near the Paleocene (Chen et al., 2016) suggests that the lack of stylets dates at least to the most recent common ancestor of all *Stomaphis*, much more than 13.8 Mya. Nevertheless, the alimentary tract still serves some purpose, e.g., its cells secrete some chemicals to the gut which are later excreted as shown by single observations of *Stomaphis graffii* male larvae extruding a droplet of fluid when disturbed (fig. 9A, B). The composition and function as well as the frequency of these excretions is unknown. It may be suspected that these may contain metabolic byproducts, because the alimentary tract in all aphids also fulfils the role of urinary system (Ponsen, 1987). The strong vacuolization of cells forming the midgut and anterior part of the hindgut suggest their intensive secretory

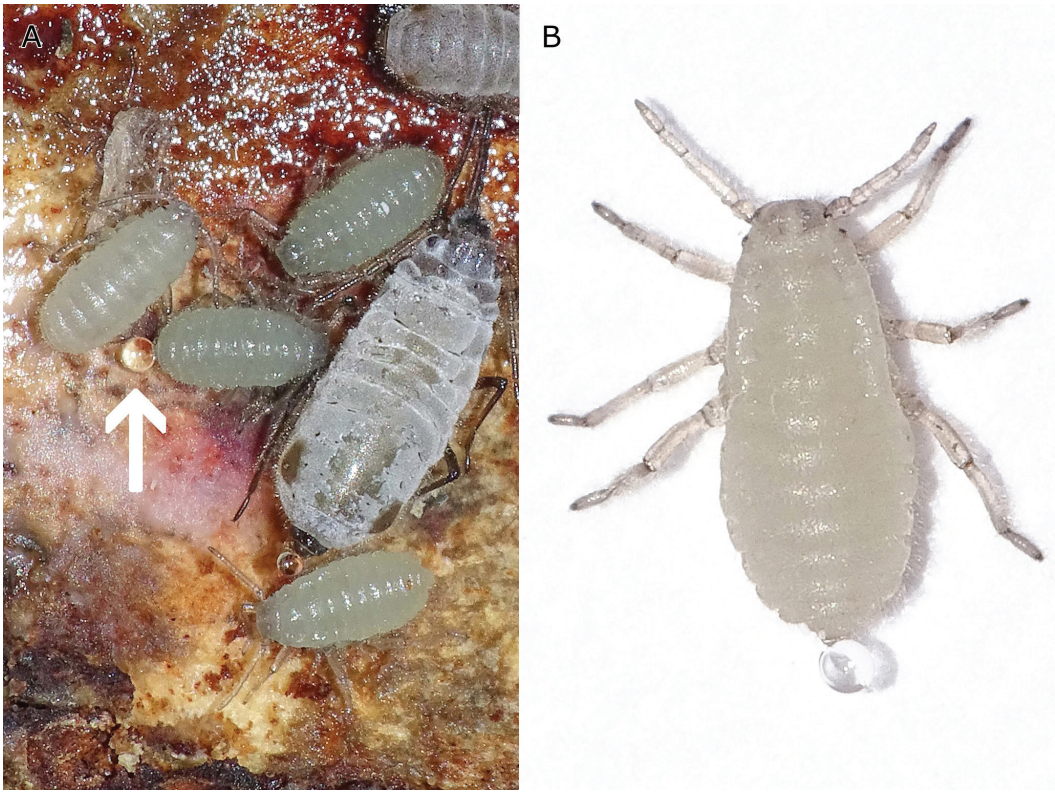

FIGURE 9 Droplets of fluid excreted by *Stomaphis graffii* males when disturbed within colony (A) and after collection (B).

activity, but the nature of their secretion is in this case unknown. Male larvae of *Stomaphis* are often observed to hide under or on females (Depa, 2013; Depa et al., 2015), so the excretion may have a defensive function against mutualist ants, if males do not produce typical honeydew. It seems then that the originally secondary or even tertiary function of this system is important enough to be positively selected and keep the existence of the system, which lost its original function. Thus, the alimentary tract may be considered an exaptation, resulting from a shift of secondary functions to role of primary function. The results of this study imply the question, to what extent are the non-primary functions of a system important enough to ensure the retention of the system under selective pressure, despite losing its original function.

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